

AMENDMENTS TO THE CLAIMS

1. (original) An isolated or recombinant nucleic acid comprising a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137 or SEQ ID NO:139, over a region of at least about 100 residues, wherein the nucleic acid encodes at least one polypeptide having a phospholipase activity, and the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection.

2-5. (canceled)

6. (original) The isolated or recombinant nucleic acid of claim 1, wherein the nucleic acid sequence encodes a polypeptide having a sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID

NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108 SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138 or SEQ ID NO:140.

7. (canceled)

8. (currently amended) The isolated or recombinant nucleic acid of claim 1, wherein the phospholipase activity comprises catalyzing hydrolysis of a glycerolphosphate ester linkage; comprises catalyzing hydrolysis of an ester linkage in a phospholipid in a vegetable oil; comprises a phospholipase C (PLC) activity; comprises a phospholipase A (PLA) activity; comprises a phospholipase B (PLB) activity; comprises a phospholipase D (PLD) activity, a phospholipase D1 or a phospholipase D2 activity; comprises hydrolysis of a glycoprotein; comprises a patatin enzymatic activity; or, comprises a lipid acyl hydrolase (LAH) activity.

9-23. (canceled)

24. (original) An isolated or recombinant nucleic acid, wherein the nucleic acid comprises a sequence that hybridizes under stringent conditions to a nucleic acid comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID

NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137 or SEQ ID NO:139, wherein the nucleic acid encodes a polypeptide having a phospholipase activity.

25-26. (canceled)

27. (original) A nucleic acid probe for identifying a nucleic acid encoding a polypeptide with a phospholipase activity, wherein the probe comprises at least 10 consecutive bases of a sequence comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137 or SEQ ID NO:139, wherein the probe identifies the nucleic acid by binding or hybridization.

28-30. (canceled)

31. (currently amended) An amplification primer sequence pair for amplifying a nucleic acid encoding a polypeptide having a phospholipase activity, wherein the primer pair is capable of amplifying a nucleic acid comprising a sequence as set forth in claim 1 or ~~claim 24~~, or a subsequence thereof.

32. (canceled)

33. (original) An amplification primer pair, wherein the primer pair comprises a first member having a sequence as set forth by about the first (the 5') 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more residues of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137 or SEQ ID NO:139, and a second member having a sequence as set forth by about the first (the 5') 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 or more residues of the complementary strand of the first member.

34. (original) A phospholipase-encoding nucleic acid generated by amplification of a polynucleotide using an amplification primer pair as set forth in claim 33.

35-37. (canceled)

38. (original) An isolated or recombinant phospholipase encoded by a phospholipase-encoding nucleic acid as set forth in claim 34.

39. (currently amended) A method of amplifying a nucleic acid encoding a polypeptide having a phospholipase activity comprising amplification of a template nucleic acid with an amplification primer sequence pair capable of amplifying a nucleic acid sequence as set forth in claim 1 ~~or claim 24~~, or a subsequence thereof.

40. (original) A method for making a phospholipase comprising amplification of a nucleic acid with an amplification primer pair as set forth in claim 33 and expression of the amplified nucleic acid.

41. (currently amended) An expression cassette comprising a nucleic acid comprising a sequence as set forth in claim 1 ~~or claim 24~~.

42. (currently amended) A vector comprising a nucleic acid comprising a sequence as set forth in claim 1 ~~or claim 24~~.

43. (currently amended) A cloning vehicle comprising a nucleic acid comprising a sequence as set forth in claim 1 ~~or claim 24~~, wherein the cloning vehicle comprises a viral vector, a plasmid, a phage, a phagemid, a cosmid, a fosmid, a bacteriophage or an artificial chromosome.

44-45. (canceled)

46. (currently amended) A transformed cell comprising a nucleic acid comprising a sequence as set forth in claim 1 ~~or claim 24~~.

47-48. (canceled)

49. (currently amended) A transgenic non-human animal comprising a sequence as set forth in claim 1 ~~or claim 24~~.

50. (canceled)

51. (currently amended) A transgenic plant comprising a sequence as set forth in claim 1 ~~or claim 24~~.

52. (canceled)

53. (currently amended) A transgenic seed comprising a sequence as set forth in claim 1 ~~or claim 24~~.

54. (canceled)

55. (currently amended) An antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a sequence as set forth in claim 1 ~~or claim 24~~, or a subsequence thereof.

56. (canceled)

57. (currently amended) A method of inhibiting the translation of a phospholipase message in a cell comprising administering to the cell or expressing in the cell an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a sequence as set forth in claim 1 ~~or claim 24~~.

58. (currently amended) A double-stranded inhibitory RNA (RNAi) molecule comprising a subsequence of a sequence as set forth in claim 1 ~~or claim 24~~.

59. (canceled)

60. (currently amended) A method of inhibiting the expression of a phospholipase in a cell comprising administering to the cell or expressing in the cell a double-stranded inhibitory RNA (iRNA), wherein the RNA comprises a subsequence of a sequence as set forth in claim 1 or ~~claim 24.~~

61 (original) An isolated or recombinant polypeptide (i) having at least 50% sequence identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, , SEQ ID NO:108 SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138 or SEQ ID NO:140, over a region of at least about 100 residues, wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection, or, (ii) encoded by a nucleic acid having at least 50% sequence identity to a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID

NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137 or SEQ ID NO:139, over a region of at least about 100 residues, and the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection, or encoded by a nucleic acid capable of hybridizing under stringent conditions to a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137 or SEQ ID NO:139.

62 to 64. (canceled)

65. (original) The isolated or recombinant polypeptide of claim 61, wherein the polypeptide has a phospholipase activity.

66. (currently amended) The isolated or recombinant polypeptide of claim 65, wherein the phospholipase activity comprises catalyzing hydrolysis of a glycerolphosphate ester linkage; comprises catalyzing hydrolysis of an ester linkage in a phospholipid in a vegetable oil; comprises a phospholipase C (PLC) activity; comprises a phospholipase A (PLA) activity; comprises a phospholipase A1 or phospholipase A2 activity; comprises a phospholipase D (PLD) activity; comprises a phospholipase D1 or a phospholipase D2 activity; comprises hydrolysis of a glycoprotein; comprises a patatin enzymatic activity; or, comprises a lipid acyl hydrolase (LAH) activity.

67 to 82. (canceled)

83. (original) An isolated or recombinant polypeptide comprising a polypeptide as set forth in claim 61 and lacking a signal sequence.

84. (original) An isolated or recombinant polypeptide comprising a polypeptide as set forth in claim 61 and having a heterologous signal sequence.

85 to 92. (canceled)

93. (original) A protein preparation comprising a polypeptide as set forth in claim 61, wherein the protein preparation comprises a liquid, a solid or a gel.

94. (currently amended) A heterodimer or homodimer comprising a polypeptide as set forth in claim 61 and a second domain.

95 to 97. (canceled)

98. (original) An immobilized polypeptide, wherein the polypeptide comprises a sequence as set forth in claim 61, or a subsequence thereof.

99. (canceled)

100. (original) An array comprising an immobilized polypeptide as set forth in claim 61.

101. (currently amended) An array comprising an immobilized nucleic acid as set forth in claim 1 ~~or claim 24~~.

102. (original) An isolated or recombinant antibody that specifically binds to a polypeptide as set forth in claim 61.

103. (canceled)

104. (original) A hybridoma comprising an antibody that specifically binds to a polypeptide as set forth in claim 61.

105. (original) A method of isolating or identifying a polypeptide with a phospholipase activity comprising the steps of:

(a) providing an antibody as set forth in claim 102;
(b) providing a sample comprising polypeptides; and
(c) contacting the sample of step (b) with the antibody of step (a) under conditions wherein the antibody can specifically bind to the polypeptide, thereby isolating or identifying a polypeptide having a phospholipase activity.

106. (currently amended) A method of making an anti-phospholipase antibody comprising administering to a non-human animal a nucleic acid as set forth in claim 1 ~~or claim 24~~ or a subsequence thereof in an amount sufficient to generate a humoral immune response, thereby making an anti-phospholipase antibody.

107. (original) A method of making an anti-phospholipase antibody comprising administering to a non-human animal a polypeptide as set forth in claim 61 or a subsequence thereof in an amount sufficient to generate a humoral immune response, thereby making an anti-phospholipase antibody.

108. (currently amended) A method of producing a recombinant polypeptide comprising the steps of: (a) providing a nucleic acid operably linked to a promoter, wherein the nucleic acid comprises a sequence as set forth in claim 1 ~~or claim 24~~; and (b) expressing the nucleic acid of step (a) under conditions that allow expression of the polypeptide, thereby producing a recombinant polypeptide.

109. (canceled)

110. (original) A method for identifying a polypeptide having a phospholipase activity comprising the following steps:

(a) providing a polypeptide as set forth in claim 65;

(b) providing a phospholipase substrate; and

(c) contacting the polypeptide with the substrate of step (b) and detecting a decrease in the amount of substrate or an increase in the amount of a reaction product, wherein a decrease in the amount of the substrate or an increase in the amount of the reaction product detects a polypeptide having a phospholipase activity.

111. (original) A method for identifying a phospholipase substrate comprising the following steps:

(a) providing a polypeptide as set forth in claim 65;

(b) providing a test substrate; and

(c) contacting the polypeptide of step (a) with the test substrate of step (b) and detecting a decrease in the amount of substrate or an increase in the amount of reaction product, wherein a

decrease in the amount of the substrate or an increase in the amount of a reaction product identifies the test substrate as a phospholipase substrate.

112. (currently amended) A method of determining whether a test compound specifically binds to a polypeptide comprising the following steps:

- (a) expressing a nucleic acid or a vector comprising the nucleic acid under conditions permissive for translation of the nucleic acid to a polypeptide, wherein the nucleic acid has a sequence as set forth in claim 1 ~~or claim 24~~;
- (b) providing a test compound;
- (c) contacting the polypeptide with the test compound; and
- (d) determining whether the test compound of step (b) specifically binds to the polypeptide.

113. (original) A method of determining whether a test compound specifically binds to a polypeptide comprising the following steps:

- (a) providing a polypeptide as set forth in claim 61;
- (b) providing a test compound;
- (c) contacting the polypeptide with the test compound; and
- (d) determining whether the test compound of step (b) specifically binds to the polypeptide.

114. (original) A method for identifying a modulator of a phospholipase activity comprising the following steps:

- (a) providing a polypeptide as set forth in claim 65;
- (b) providing a test compound;
- (c) contacting the polypeptide of step (a) with the test compound of step (b) and measuring an activity of the phospholipase, wherein a change in the phospholipase activity measured in the presence of the test compound compared to the activity in the absence of the test compound provides a determination that the test compound modulates the phospholipase activity.

115-117. (canceled)

118. (currently amended) A computer system or a computer readable medium comprising a processor and a data storage device wherein said data storage device or the computer readable medium has stored thereon a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises sequence as set forth in claim 61, a polypeptide encoded by a nucleic acid as set forth in claim 1 ~~or claim 24~~.

119 to 122. (canceled)

123. (currently amended) A method for identifying a feature in a sequence comprising the steps of: (a) reading the sequence using a computer program which identifies one or more features in a sequence, wherein the sequence comprises a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises a polypeptide as set forth in claim 61; a polypeptide encoded by a nucleic acid as set forth in claim 1 ~~or claim 24~~; and (b) identifying one or more features in the sequence with the computer program.

124-127. (canceled)

128. (original) A method for isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from an environmental sample comprising the steps of:

- (a) providing an amplification primer sequence pair as set forth in claim 33;
- (b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to the amplification primer pair; and,
- (c) combining the nucleic acid of step (b) with the amplification primer pair of step (a) and amplifying nucleic acid from the environmental sample, thereby isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from an environmental sample.

129. (canceled)

130. (currently amended) A method for isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from an environmental sample comprising the steps of:

(a) providing a polynucleotide probe comprising a sequence as set forth in claim 1 or ~~claim 24~~, or a subsequence thereof;

(b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to a polynucleotide probe of step (a);

(c) combining the isolated nucleic acid or the treated environmental sample of step (b) with the polynucleotide probe of step (a); and

(d) isolating a nucleic acid that specifically hybridizes with the polynucleotide probe of step (a), thereby isolating or recovering a nucleic acid encoding a polypeptide with a phospholipase activity from an environmental sample.

131-132. (canceled)

133. (currently amended) A method of generating a variant of a nucleic acid encoding a polypeptide with a phospholipase activity comprising the steps of:

(a) providing a template nucleic acid comprising a sequence as set forth in claim 1 or ~~claim 24~~; and

(b) modifying, deleting or adding one or more nucleotides in the template sequence, or a combination thereof, to generate a variant of the template nucleic acid.

134-142. (canceled)

143. (currently amended) A method for modifying codons in a nucleic acid encoding a polypeptide with a phospholipase activity to increase its expression in a host cell, the method comprising the following steps:

(a) providing a nucleic acid encoding a polypeptide with a phospholipase activity comprising a sequence as set forth in claim 1 or ~~claim 24~~; and,

(b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.

144. (currently amended) A method for modifying codons in a nucleic acid encoding a phospholipase polypeptide, the method comprising the following steps:

(a) providing a nucleic acid encoding a polypeptide with a phospholipase activity comprising a sequence as set forth in claim 1 or ~~claim 24~~; and,

(b) identifying a codon in the nucleic acid of step (a) and replacing it with a different codon encoding the same amino acid as the replaced codon, thereby modifying codons in a nucleic acid encoding a phospholipase.

145. (currently amended) A method for modifying codons in a nucleic acid encoding a phospholipase polypeptide to increase its expression in a host cell, the method comprising the following steps:

(a) providing a nucleic acid encoding a phospholipase polypeptide comprising a sequence as set forth in claim 1 or ~~claim 24~~; and,

(b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding

sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.

146. (currently amended) A method for modifying a codon in a nucleic acid encoding a polypeptide having a phospholipase activity to decrease its expression in a host cell, the method comprising the following steps:

(a) providing a nucleic acid encoding a phospholipase polypeptide comprising a sequence as set forth in claim 1 ~~or claim 24~~; and

(b) identifying at least one preferred codon in the nucleic acid of step (a) and replacing it with a non-preferred or less preferred codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in a host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to decrease its expression in a host cell.

147. (canceled)

148. (original) A method for producing a library of nucleic acids encoding a plurality of modified phospholipase active sites or substrate binding sites, wherein the modified active sites or substrate binding sites are derived from a first nucleic acid comprising a sequence encoding a first active site or a first substrate binding site the method comprising the following steps:

(a) providing a first nucleic acid encoding a first active site or first substrate binding site, wherein the first nucleic acid sequence comprises a sequence that hybridizes under stringent conditions to a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55,

SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137 or SEQ ID NO:139, or a subsequence thereof, and the nucleic acid encodes a phospholipase active site or a phospholipase substrate binding site;

(b) providing a set of mutagenic oligonucleotides that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and,

(c) using the set of mutagenic oligonucleotides to generate a set of active site-encoding or substrate binding site-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized, thereby producing a library of nucleic acids encoding a plurality of modified phospholipase active sites or substrate binding sites.

149-151. (canceled)

152. (currently amended) A method for making a small molecule comprising the following steps:

(a) providing a plurality of biosynthetic enzymes capable of synthesizing or modifying a small molecule, wherein one of the enzymes comprises a phospholipase enzyme encoded by a nucleic acid comprising a sequence as set forth in claim 1 ~~or claim 24~~;

(b) providing a substrate for at least one of the enzymes of step (a); and

(c) reacting the substrate of step (b) with the enzymes under conditions that facilitate a plurality of biocatalytic reactions to generate a small molecule by a series of biocatalytic reactions.

153. (currently amended) A method for modifying a small molecule comprising the following steps:

(a) providing a phospholipase enzyme, wherein the enzyme comprises a polypeptide as set forth in claim 65, or a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence as set forth in claim 1 ~~or claim 24~~;

(b) providing a small molecule; and

(c) reacting the enzyme of step (a) with the small molecule of step (b) under conditions that facilitate an enzymatic reaction catalyzed by the phospholipase enzyme, thereby modifying a small molecule by a phospholipase enzymatic reaction.

154-157. (canceled)

158. (currently amended) A method for determining a functional fragment of a phospholipase enzyme comprising the steps of:

(a) providing a phospholipase enzyme, wherein the enzyme comprises a polypeptide as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 ~~or claim 24~~;

and

(b) deleting a plurality of amino acid residues from the sequence of step (a) and testing the remaining subsequence for a phospholipase activity, thereby determining a functional fragment of a phospholipase enzyme.

159. (canceled)

160. (currently amended) A method for whole cell engineering of new or modified phenotypes by using real-time metabolic flux analysis, the method comprising the following steps:

(a) making a modified cell by modifying the genetic composition of a cell, wherein the genetic composition is modified by addition to the cell of a nucleic acid comprising a sequence as set forth in claim 1 ~~or claim 24~~;

(b) culturing the modified cell to generate a plurality of modified cells;

(c) measuring at least one metabolic parameter of the cell by monitoring the cell culture of step (b) in real time; and,

(d) analyzing the data of step. (c) to determine if the measured parameter differs from a comparable measurement in an unmodified cell under similar conditions, thereby identifying an engineered phenotype in the cell using real-time metabolic flux analysis.

161-163. (canceled)

164. (original) An isolated or recombinant signal sequence consisting of a sequence as set forth in residues 1 to 16, 1 to 17, 1 to 18, 1 to 19, 1 to 20, 1 to 21, 1 to 22, 1 to 23, 1 to 24, 1 to 25, 1 to 26, 1 to 27, 1 to 28, 1 to 28, 1 to 30 or 1 to 31, 1 to 32 or 1 to 33 of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID

NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108 SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:132, SEQ ID NO:134, SEQ ID NO:136, SEQ ID NO:138 or SEQ ID NO:140.

165. (original) A chimeric polypeptide comprising at least a first domain comprising signal peptide (SP) having a sequence as set forth in claim 164, and at least a second domain comprising a heterologous polypeptide or peptide, wherein the heterologous polypeptide or peptide is not naturally associated with the signal peptide (SP).

166-167. (canceled)

168. (currently amended) An isolated or recombinant nucleic acid encoding a chimeric polypeptide, wherein the chimeric polypeptide comprises at least a first domain comprising signal peptide [(SP)] (SP) having a sequence as set forth in claim 164 and at least a second domain comprising a heterologous polypeptide or peptide, wherein the heterologous polypeptide or peptide is not naturally associated with the signal peptide (SP).

169. (currently amended) A method of increasing thermotolerance or thermostability of a phospholipase polypeptide, the method comprising glycosylating a phospholipase, wherein the polypeptide comprises at least thirty contiguous amino acids of a polypeptide as set forth in claim

61, or a polypeptide encoded by a nucleic acid as set forth in claim 1 ~~or claim 24~~, thereby increasing the thermotolerance or thermostability of the phospholipase.

170. (currently amended) A method for overexpressing a recombinant phospholipase in a cell comprising expressing a vector comprising a nucleic acid sequence as set forth in claim 1 ~~or claim 24~~, wherein overexpression is effected by use of a high activity promoter, a dicistronic vector or by gene amplification of the vector.

171. (currently amended) A method of making a transgenic plant comprising the following steps:

(a) introducing a heterologous nucleic acid sequence into the cell, wherein the heterologous nucleic sequence comprises a sequence as set forth in claim 1 ~~or claim 24~~, thereby producing a transformed plant cell;

(b) producing a transgenic plant from the transformed cell.

172-173. (canceled)

174. (currently amended) A method of expressing a heterologous nucleic acid sequence in a plant cell comprising the following steps:

(a) transforming the plant cell with a heterologous nucleic acid sequence operably linked to a promoter, wherein the heterologous nucleic sequence comprises a sequence as set forth in claim 1 ~~or claim 24~~;

(b) growing the plant under conditions wherein the heterologous nucleic acids sequence is expressed in the plant cell.

175. (currently amended) A method for hydrolyzing, breaking up or disrupting a phospholipid-comprising composition comprising the following steps:

(a) providing a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 ~~or claim 24~~;

(b) providing a composition comprising a phospholipid; and

(c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the phospholipase hydrolyzes, breaks up or disrupts the phospholipid-comprising composition.

176-177. (canceled)

178. (currently amended) A method for liquefying or removing a phospholipid-comprising composition comprising the following steps:

(a) providing a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 ~~or claim 24~~;

(b) providing a composition comprising a phospholipid; and

(c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the phospholipase removes or liquefies the phospholipid-comprising composition.

179-180. (canceled)

181. (currently amended) A detergent composition comprising a polypeptide as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 ~~or claim 24~~, wherein the polypeptide has a phospholipase activity.

182. (currently amended) A method for washing an object comprising the following steps:

(a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 ~~or claim 24~~;

(b) providing an object; and

(c) contacting the polypeptide of step (a) and the object of step (b) under conditions wherein the composition can wash the object.

183. (currently amended) A method for degumming an oil comprising the following steps:

(a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 ~~or claim 24~~;

(b) providing an composition comprising an phospholipid-containing fat or oil; and

(c) contacting the polypeptide of step (a) and the composition of step (b) under conditions wherein the polypeptide can catalyze the hydrolysis of a phospholipid in the composition.

184-196. (canceled)

197. (currently amended) A method for converting a non-hydratable phospholipid to a hydratable form comprising the following steps:

(a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 ~~or claim 24~~;

(b) providing an composition comprising a non-hydratable phospholipid; and

(c) contacting the polypeptide of step (a) and the composition of step (b) under conditions wherein the polypeptide converts the non-hydratable phospholipid to a hydratable form.

198-199. (canceled)

200. (currently amended) A method for caustic refining of a phospholipid-containing composition comprising the following steps:

(a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 ~~or claim 24~~;

(b) providing an composition comprising a phospholipid; and

(c) contacting the polypeptide of step (a) with the composition of step (b) before, during or after the caustic refining.

201-204. (canceled)

205. (currently amended) A method for purification of a phytosterol or a triterpene comprising the following steps:

(a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 ~~or claim 24~~;

(b) providing an composition comprising a phytosterol or a triterpene; and

(c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the polypeptide can catalyze the hydrolysis of a phospholipid in the composition.

206-211. (canceled)

212. (currently amended) A method for refining a crude oil comprising the following steps:

(a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 ~~or claim 24~~;

(b) providing a composition comprising an oil comprising a phospholipid; and

(c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the polypeptide can catalyze the hydrolysis of a phospholipid in the composition.

213-226. (canceled)

227. (currently amended) A method for degumming an oil or a fat comprising the following steps:

(a) providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 ~~or claim 24~~, wherein the phospholipase activity comprises a phospholipase D activity, and a phosphatase enzyme;

(b) providing an composition comprising an phospholipid-containing fat or oil; and

(c) contacting the polypeptide of step (a) and the composition of step (b) under conditions wherein the polypeptide can catalyze the hydrolysis of a phospholipid in the composition.

228. (currently amended) A composition having the equivalent of a phospholipase C activity comprising providing a composition comprising a polypeptide having a phospholipase activity as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 ~~or claim 24~~, wherein the phospholipase activity comprises a phospholipase D activity, and a phosphatase enzyme.

229. (currently amended) A method for ameliorating or preventing lipopolysaccharide (LPS)-mediated toxicity comprising administering to a patient a pharmaceutical composition comprising a polypeptide as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 ~~or claim 24~~.

230. (currently amended) A method for detoxifying an endotoxin comprising contacting the endotoxin with a polypeptide as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 ~~or claim 24~~.

231. (currently amended) A method for deacylating a 2' or a 3' fatty acid chain from a lipid A comprising contacting the lipid A with a polypeptide as set forth in claim 65, or a polypeptide encoded by a nucleic acid as set forth in claim 1 ~~or claim 24~~.

232-233. (canceled)